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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Doherty et al.
Filing Date: 20 January 1999
Serial No.: 09/234,208
For: HER-2 BINDING ANTAGONISTS
Art Unit: 1642
Examiner: Jennifer Hunt
Docket: 49321-1
Date: 18 November 2002

Commissioner for Patents
Washington, DC 20231

AFFIDAVIT OF DR. EDWARD NEUWELT UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF AMENDMENT B UNDER37 CFR § 1.111)

Sir or Madam:

I, Dr. Edward Neuwelt, being duly sworn, say:

1. I am an internationally recognized scientist and am presently employed as a professor of neurosurgery and neurology in the Oregon Health and Sciences University School of Medicine and the Portland Veterans Affairs Medical Center in Portland, Oregon (from 1981 to present). I am also the director of the Blood Brain Barrier Program at Oregon Health and Science University and the recipient, this year, of the unique $3.7 million Javitz Neuroscience Investigator Award. I received an M.D. degree from the University of Colorado, Denver, CO in 1972 (graduating Magna cum Laude), with an internship at University of Colorado, Denver, CO in 1973, specializing in Straight Surgery and completed residency training specializing in neurosurgery at University of Texas, Dallas, Texas in 1978.

2. I am an author or co-author of more than one hundred and eighty (180) peer-reviewed research articles and I am a member of a number of scientific and medical societies, most notably AANS, CNE, ASCO and AACR. I have received a number of prizes and awards

for achievement in research, most notably I was the recipient of a Javitz Discovery Award, and numerous grants from the federal government, including NIH and VA. I have served on several peer review groups and study sections and have been invited to give numerous presentations on my research at national and international meetings.

3. I have read the above-identified patent application, and understand that particular claims have been rejected under 35 U.S.C. § 112, ¶ 1, based on an alleged lack of enablement for *in vivo* utility. In particular, it appears that the Office does not fully appreciate the therapeutic significance, including the *in vivo* therapeutic significance, of the anchorage-independent (in soft agar) cancer cell growth experiments described at page 13 of the originally filed patent application involving SKOV-3 and 17-3-1 carcinoma cells. While the disclosed experiments of the application are *in vitro* cell culture experiments, the soft agar assay utilized is a widely recognized model system for human cancer (DiFore et al., *Science* 237:178-182, 1987; Hudziak et al., *Proc. Natl. Acad. Sci. USA* 84:7159-7163, 1987; and Baasner et al., *Oncogene* 13:901-911, 1996; all cited in said patent application and attached hereto as **EXHIBIT A**), and thus inhibition of such anchorage-independent growth in this system should be, and is within the relevant art taken as substantial proof of not only a well-established *in vivo* utility, but also a specific, credible and substantial *in vivo* utility. This is especially true where the therapeutic *target* HER-2 receptor is already a ***bona fide* clinical target** of the Herceptin™ the FDA-approved humanized monoclonal antibody, and where herstatin, unlike Herceptin™, is a naturally occurring protein having a high degree of specificity, and binding affinity.

4. Nonetheless, I have conducted and supervised, in collaboration with Dr. Gail Clinton who is an original and true inventor of the subject matter of the above-identified patent application, a series of concurrent tests using techniques and cell lines that were available in the art at the time of filing of said patent application, to further confirm, illustrate and support the *in vivo* therapeutic efficacy of herstatin as originally disclosed and enabled by the soft agar cell growth experiments therein. The instant experiments were conducted using the art-recognized *in vivo* human tumor model; namely, human U87MG injected into nude rats (*see e.g.*, O'Rourke et al. *Proc. Natl. Acad. Sci. USA*, 94:3250-3255, 1997, entitled "Trans receptor inhibition of human glioblastoma cells by erbB family ectodomains"; attached hereto as **EXHIBIT B**). U87MG is a

standard human glioblastoma cancer cell line that expresses HER-2 and the EGF receptor (*Id*). Significantly, human glioblastoma is typically an aggressive cancer, and is refractory to therapy. The data is summarized in the following Table:

TABLE 1: Herstatin expression inhibited tumor growth and significantly enhanced survival of rats with intra cerebral U87 gliomas.

Animal Number	Cell line	Status (days post-cell injection)
G9	U87MG	Significant weight loss on day 20; euthanized on day 25
G10	U87MG	Significant weight loss on day 20; euthanized on day 25
G11	U87MG	Significant weight loss on day 16; euthanized on day 19
G12	U87MG	Significant weight loss on day 20; euthanized on day 26
G13	U87MG/Hst	Normal condition throughout; sacrificed at day 57 with no signs of neurotoxicity
G14	U87MG/Hst	Normal condition throughout; sacrificed at day 57 with no signs of neurotoxicity
G15	U87MG/Hst	Normal condition throughout; sacrificed at day 57 with no signs of neurotoxicity
G16	U87MG/Hst	Normal condition throughout; sacrificed at day 57 with no signs of neurotoxicity

Briefly, either "U87MG/Hst" cells (from a herstatin-expressing U87MG cell line derived from U87MG glioblastoma cells stably transfected with herstatin), or control U87MG cells, were injected into the brains of nude rats. All rats receiving U87MG cells exhibited severe weight loss by 20 days post-injection and were necessarily euthanized because of tumor burden; all having large tumors (about 150 mm^3) in the right cerebral hemisphere. By contrast, rats receiving U87MG/Hst cells were all still alive and healthy at 57 days post-inoculation and were

then sacrificed to assess for the presence of any tumor growth. **No tumor growth was found in any of the U87MG/Hst animals**, but herstatin immunostaining was membrane localized on cells in the brain surface at the inoculation site. Additionally, implantation of a second U87MG/Hst cell line having relatively low herstatin expression, resulted in significantly reduced intracerebral tumor volume, compared with parental U87MG xenografts. Significantly, U87MG/Hst and U87MG cells have comparable growth rates in culture.

Significantly, the above results are entirely consistent with those previously disclosed in the above-identified pending patent application. These results are particularly probative and compelling in view of the fact that by far the greatest percentage of brain tumors arise by metastasis of breast or lung cancer cells, both cell types being among the valid and preferred therapeutic targets of herstatin, as taught, disclosed and enabled in said patent application.

For the experiments of the above Table, the herstatin-expressing U87MG cell line ("U87MG/Hst") was derived by selection (hygromycin at 0.5 mg/ml) of U87MG cells transfected with the 5.6 Kb herstatin cDNA expression vector pcDNA3.1(-)/Hygromycin (based on an expression vector from Invitrogen) (*see* **EXHIBIT C**, attached hereto, for vector map). The expression vector drives herstatin expression from the CMV promoter, and uses the herstatin signal peptide sequence to insure proper trafficking and secretion. Prior to injection into rat brains (1×10^6 live cells/rat), the U87MG/Hst and U87MG cells were grown in DMEM (10% fbs) in 15-cm plates. Animal weights were monitored daily.

5. In conclusion, the art-recognized U87MG human cancer model system, available in the art at the time of filing of the above-identified pending patent application, has been used to further confirm, illustrate and support the *in vivo* therapeutic utility of herstatin as originally taught, disclosed and enabled by said patent application, base therein on an art-recognized and accepted *in vitro* soft-agar cell growth system with appropriate human cancer cells. **Herstatin expression inhibited tumor growth and significantly enhanced survival of rats with intra cerebral U87 gliomas**.

6. I further declare that all statements made herein of my own knowledge are true and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Edward Neuwelt

State of Oregon)
) ss.:
County of Multnoma)

On this 18 th day of November, 2002, before me, a Notary Public in and for the State and County aforesaid, personally appeared Edward Neuwelt, to me known and known to me to be the person of that name, who signed and sealed the foregoing instrument, and he acknowledged the same to be her free act and deed.

Notary Public

OFFICIAL SEAL
AMY HUDDLESTON
NOTARY PUBLIC - OREGON
COMMISSION NO. 325299
MY COMMISSION EXPIRES JUL 15, 2003

Commission expires July 15, 2003